Clinical Utility of Epidermal Growth Factor Receptor Expression for Selecting Patients with Advanced Non-small Cell Lung Cancer for Treatment with Erlotinib

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Introduction: Erlotinib (Tarceva®) has demonstrated a survival benefit in unselected patients with advanced non-small cell lung cancer (NSCLC) after failure of chemotherapy. Because not all patients benefit from erlotinib, epidermal growth factor receptor (EGFR) protein expression may provide a basis for selecting patients for treatment with this EGFR inhibitor.

Methods: Tumor samples from patients who participated in National Institute of Canada Clinical Trials Group Study BR.21 were assayed by immunohistochemistry using Dako EGFR pharmDx™ kits. EGFR expression was scored as proportion of tumor cells with membrane staining, staining intensity, and combined proportion and intensity scores. All possible cutpoints were examined to determine whether EGFR protein expression status by immunohistochemistry might be useful for predicting patient survival.

Results: Three hundred twenty-five patients had evaluable assay results. The prognostic significance of EGFR protein expression was modest. Patients with EGFR-positive tumors who received placebo after failure of chemotherapy had slightly worse survival than patients with EGFR-negative tumors; however, the differences were not statistically significant for any cutpoint for any of the three measures of EGFR protein expression. The treatment benefits from erlotinib relative to placebo were greater for EGFR-positive patients compared with EGFR-negative patients, but they were not significantly different for any cutoff used to define EGFR positivity. Use of very high cutpoints to define patients with EGFR-rich tumors that might be especially sensitive to treatment with erlotinib cannot be supported by these data.

Conclusions: Selection or exclusion of NSCLC patients for erlotinib therapy after failure of standard therapy for advanced or metastatic disease should not be based solely on EGFR protein expression as determined by immunohistochemistry.

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The epidermal growth factor receptor (EGFR) pathway is involved in cell proliferation, metastasis, and angiogenesis, and components of this pathway have been implicated in many different cancer types as a promoter of tumor growth. Elevated levels of EGFR determined by immunohistochemistry (IHC) have been correlated with poor clinical outcomes of patients with head and neck, ovarian, cervical, bladder, and esophageal cancer, whereas more modest relationships have been reported for gastric, breast, endometrial, and colorectal cancers.[1] The prognostic role of EGFR expression in non-small cell lung cancer (NSCLC) is unclear. Some studies have reported relationships between high expression of EGFR and poor clinical outcomes,[2–4] including a meta-analysis of 16 studies.[5] However, other studies have failed to find any relationships.[6–8]

The emergence of therapies that specifically target the EGFR pathway, either antibodies or small-molecule inhibitors of the EGFR tyrosine kinase, raises questions about the predictive utility of EGFR for patients with NSCLC who are treated with these EGFR inhibitors. Most reported studies have found no relationships between EGFR expression by IHC and clinical outcomes of patients treated with gefinitib (AstraZeneca, Wilmington, DE)[9–13] or erlotinib (OSI Pharmaceuticals, Inc., Melville, NY),[14–16] although none of these studies included an untreated control arm. Relationships with gefitinib have been reported in two recent studies using relatively high cutoffs to define EGFR positivity.[17–18]

The terms "prognostic" and "predictive" have been used in numerous publications to describe relationships between biomarkers and clinical outcomes; however, these terms are seldom defined and are often used interchangeably. In this assessment, we will use the definitions proposed by Clark[19,20] and Hayes.[21] A prognostic factor is a measurement that is associated with clinical outcome in the absence of therapy or with the application of a standard therapy that all patients are likely to receive. It can be thought of as a measure of the natural history of the disease. A control group from a randomized clinical trial is an ideal setting for evaluating the prognostic significance of a biomarker. A predictive factor is a measurement that is associated with response or lack of response to a particular therapy. Response can be defined using any of the clinical endpoints commonly used in clinical trials. A predictive factor implies a differential benefit from

the therapy that depends on the status of the predictive biomarker. In statistical terms, this constitutes an interaction between treatment benefit and biomarker status that is best evaluated in a randomized clinical trial with a control group.

National Institute of Canada Clinical Trials Group (NCIC CTG) Study BR.21 was a randomized, placebo-controlled study of erlotinib (Tarceva®, OSI Pharmaceuticals, Inc., Melville, NY) versus placebo for the second- or third-line treatment of patients with advanced NSCLC. The placebo control arm provided a unique opportunity to evaluate the prognostic significance of EGFR expression by IHC in these patients, and the randomization between placebo and single-agent erlotinib permitted assessment of the predictive significance of EGFR expression in this setting. Treatment with single-agent erlotinib eliminates the confounding effects of chemotherapy and/or radiotherapy that may be present when EGFR inhibitors are combined with other treatment modalities.

Evaluation of the clinical significance of EGFR expression by IHC has been complicated by the use of different antibodies, different scoring systems, and different clinical endpoints. In this report, we focus on one diagnostic kit (Dako EGFR pharmDx™ kit) and one clinical outcome (survival) from NCIC CTG Study BR.21 to determine an optimal scoring system, including appropriate cutpoints, for assessing the prognosis of patients with NSCLC and for predicting treatment benefit from erlotinib.

MATERIALS AND METHODS

Erlotinib (Tarceva®, OSI-774)

Erlotinib is a potent, reversible, EGFR tyrosine kinase inhibitor that is administered orally. It was approved in the United States in November of 2004 for the treatment of locally advanced or metastatic NSCLC after the failure of at least one prior chemotherapy regimen, based on the results from NCIC CTG Study BR.21 and supporting studies. The recommended dosing regimen is 150 mg orally once daily on a continuous schedule.[21]

Patients

NCIC CTG Study BR.21 has been previously described.[22] Briefly, 731 patients with incurable stage IIIB/IV NSCLC after failure of at least one, but no more than two prior regimens for advanced or metastatic disease were stratified by center, performance status, best response to prior therapy, number of prior regimens, and exposure to prior platinum, and were randomized 2:1 to receive 150 mg daily of erlotinib or placebo. The primary endpoint of the study was overall survival. A total of 587 patients (80%) had died at the time of database lock, and the median follow-up of patients still alive was 15 months (range, 0.4–26 months). Treatment with erlotinib produced a statistically significant improvement in both survival and progression-free survival. Assessment of EGFR status was not a requirement for study participation. Submission of tumor samples was voluntary and required a separate informed consent.

EGFR IHC Assays

Biopsy specimens were collected from patients who gave informed consent and were stored as paraffin blocks or unstained slides. These specimens were archival tumor samples and were usually obtained at the time of initial diagnosis. Slides were prepared by personnel in the NCIC Tumor Bank and were sent to LabCorp (Research Triangle Park, NC), where EGFR protein expression was determined using the Dako EGFR pharmDx™ IHC kits (DakoCytomation California Inc, Carpinteria, CA). Two pathologists trained to interpret the Dako pharmDX staining and certified by Dako onsite training interpreted the staining results. The majority of the interpretations were performed by one of the pathologists, but the other pathologist provided coverage when necessary. Slides were sent with a masked identification number so that all assays would be performed blinded to patient characteristics, treatment assignment, and clinical outcome. Analyses were batched when possible in groups of approximately 50 samples. Scoring was performed according to the manufacturer's instructions: 1) assess the total percentage of tumor cells showing membrane staining (any intensity) with EGFR (0–100%) (proportion score, PS); 2) assess the highest intensity of membrane staining in EGFR stained cells (0 = no staining, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining) (intensity score, IS); 3) assess the percentage of cells displaying the highest intensity of membrane staining (0–100%); 4) describe membrane staining as predominantly complete or incomplete; and 5) assess the percentage of cytoplasmic staining observed (0–100%). Endpoints 3, 4, and 5 were not evaluated as prognostic or predictive factors in this study. Any and all areas of the tumor were assessed for whether there was staining, whether the staining was membrane staining, the intensity of the staining, and the completeness of the staining. All staining runs were accompanied by the cell-line control slide that is included with the kit; in addition, each staining run had two additional "tumor control" slides containing sections of formalin-fixed, paraffin-embedded tumors that had been previously scored as "0" and "1+, weak" intensity. An assay was successful if tumor was present in the section and if all of the controls showed staining similar to the intended staining. Any section that showed no staining of tumor cells and no internal control positive staining was repeated. Any section that showed no staining of tumor cells but had positive internal control staining of benign structures (e.g., perineural locations) was interpreted, with a comment indicating the presence of internal control staining.[23]

There is no consensus in the literature about how to summarize these scoring assessments into a single determination of EGFR protein expression status as EGFR positive or EGFR negative. The Dako Cytomation EGFR pharmDx Interpretation Manual suggests that any membrane staining should be considered positive.[24] The statistical analysis plan for NCIC CTG Study BR.21 prespecified membrane staining in at least 10% of tumor cells for a sample to be declared EGFR positive, and clinical correlations have been published using this definition.[25] Some studies have focused on the

intensity of the staining,[14] and others have used a hybrid of the PS and the intensity.[17,18]

Statistical Methods

Three measures of EGFR positivity were evaluated: a) PS, defined as the percentage of tumor cells stained (any intensity) with EGFR; b) IS, defined as the highest intensity of membrane staining in EGFR-stained cells (0 = no staining, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining); c) hybrid score (HS), defined as PS multiplied by (IS +1), yielding a HS ranging from 0 to 400.[17]

Survival benefit was summarized using the hazard ratio, defined as the ratio of the death rates among patients still alive in two subsets at each point in time. Under the assumption of proportional hazards, the ratio is the same at each point in time. Hazard ratios were estimated from Cox proportional hazards models as implemented in PROC PHREG in SAS, Version 8.2 (Cary, NC).

To assess the prognostic significance of each of these EGFR scores, only patients from the placebo arm of NCIC CTG Study BR.21 were included in the analyses. A search for the "optimal" cutpoint was performed by classifying patients as high or low EGFR using every possible cutpoint for each of the three measures, creating a univariate Cox model for each cutpoint, and calculating the resulting hazard ratio for death for patients with EGFR-positive tumors relative to patients with EGFR-negative tumors. "Optimal" was defined as the cutpoint that yielded the smallest p value from a log-rank test. Because several published studies assessed the prognostic significance of EGFR expression in patients uniformly treated with an EGFR inhibitor, similar analyses were performed using only patients in the erlotinib arm.

To assess the predictive significance of each of these EGFR scores, all patients with evaluable EGFR assays were included in the analyses. Separate Cox models were constructed for EGFR-positive patients and for EGFR-negative patients to estimate treatment effects of erlotinib relative to placebo for each cutpoint of each EGFR measure. A series of Cox models were then created that included factors for treatment (erlotinib versus placebo), EGFR status (high versus low), and the interaction between treatment and EGFR status. The p values associated with the Wald statistic for the interaction term from the full models containing all three factors and the corresponding hazard ratio for death were evaluated to determine whether any cutpoints produced statistically significant interactions indicative of differential predictive effects depending on EGFR status.

All statistical analyses were performed by the sponsor of the clinical trial and were confirmed by NCIC CTG.

RESULTS

Tumor samples from 375 patients were sent to the central laboratory for EGFR assays by IHC, and evaluable assay results were obtained for 325 patients (87%). Among the 50 patients with unevaluable results, 38 (76%) had insufficient tumor cells in their tumor sample, six (12%) had extensive necrosis, three (6%) had inadequate control staining, two (4%) had poor tumor preservation, and one (2%) had a broken slide.

Tumors from 229 (70%) of the 325 patients with evaluable assay results had some EGFR staining. Patient demographics, tumor characteristics, and treatment outcomes for patients with or without evaluable EGFR results are displayed in Table 1. Relatively few Asian patients provided tumor samples for EGFR evaluation. Among patients with known EGFR protein expression status, there were fewer patients with Eastern Cooperative Oncology Group performance status 0 or 1, and more with two or more prior therapies, although fewer received taxanes. There was a greater incidence of nonprogression (complete response/partial response/stable disease) as the best response to prior therapy, and a longer time between diagnosis and randomization on NCIC CTG Study BR.21 was found for patients with EGFR results compared with patients without results. Despite these imbalances, no significant differences in survival were observed between patients with or without EGFR results. Most importantly, the benefit from erlotinib was similar in the two subsets.

Prognostic Significance

A total of 115 patients in the placebo arm had evaluable EGFR protein expression results by IHC, and 99 had died at the time of database lock. Univariate Cox models with EGFR results expressed as continuous variables produced hazard ratios of death greater than 1.0 (indicating worse survival for patients with increasing levels of EGFR) for each of the EGFR measures (HR = 1.008, 1.285, and 1.002 for PS, IS,

TABLE 1. Demographic and Tumor Characteristics of Patients with and without Epidermal Growth Factor Receptor (EGFR) Expression Status

Factor	Known EGFR (n = 325)	Unknown EGFR (n = 406)	p value
Erlotinib	65%	68%	0.30
Male	65%	65%	0.88
Asian	6%	17%	<0.0001
Never smoked	22%	21%	0.71
Adenocarcinoma	50%	50%	0.82
ECOG performance status 0-1	62%	70%	0.041
CR/PR/SD as best response to prior therapy	83%	76%	0.022
2+ prior regimens	57%	45%	0.0014
Prior platinum	93%	93%	1.00
Prior taxane	32%	40%	0.037
Median age (yr)	61	61	0.68
Median months since diagnosis	14	12	0.015
Median survival for erlotinib (months)	7.1	6.3	0.96
Median survival for placebo (months)	4.1	5.5	0.87
Hazard ratio for death	0.76 (p = 0.030)	0.77 (p = 0.031)	

ECOG, Eastern Cooperative Oncology Group; CR/PR/SD, complete response/partial response/stable disease.

and HS, respectively), but none were statistically significant ($p = 0.5587$, 0.3241, and 0.4702, respectively). Hazard ratios of death for EGFR-positive patients relative to EGFR-negative patients and corresponding p values for every possible cutpoint are displayed in Table 2 for each EGFR measure. Hazard ratios were greater than 1.0 for every cutpoint for each EGFR measure, but none were statistically significant ($p > 0.20$), even without adjustments for multiple-hypothesis testing. Patients with very high EGFR expression did not appear to have worse survival than patients with lower EGFR expression. The largest hazard ratios were produced when any staining was considered EGFR positive.

A total of 210 patients in the erlotinib arm had evaluable EGFR protein expression results, and 164 had died at the time of database lock. Univariate Cox models with EGFR results expressed as continuous variables produced hazard ratios very close to 1.0 for each of the EGFR measures (HR = 1.000, 1.001, and 1.000 for PS, IS, and HS, respectively), and none were statistically significant ($p = 0.9812$, 0.9908, and 0.8782, respectively). Hazard ratios and corresponding p values for every possible cutpoint are displayed in Table 2 for each EGFR measure. Hazard ratios were numerically less than 1.0 only for low cutpoints for each EGFR measure, but none were statistically significant ($p > 0.20$), even without adjustments for multiple-hypothesis testing. Patients with very high EGFR expression had slightly worse survival than patients with lower EGFR expression, as evidenced by hazard ratios greater than 1.0.

These results suggest that the prognostic significance of EGFR expression by IHC for patients with advanced NSCLC is modest at best. After failure of first-line chemotherapy, patients with EGFR-positive tumors had slightly worse survival than patients with EGFR-negative tumors if they received no additional therapy. If these patients received erlotinib, survival was slightly better for patients with EGFR-positive tumors, provided that EGFR positivity is defined using a relatively low cutpoint. However, the differences in survival were not statistically different for any cutpoint for any of the three measures of EGFR expression.

Predictive Significance

Hazard ratios for death of patients on the erlotinib arm relative to patients on the placebo arm and corresponding p values for patients with EGFR-positive tumors using every possible cutpoint are displayed in Table 3 for each EGFR measure. These hazard ratios were generally less than 1.0, regardless of the cutpoint used to define EGFR positivity, indicating a survival benefit for patients with EGFR-positive tumors who received erlotinib compared with placebo. Hazard ratios that were statistically significantly less than 1.0, with unadjusted p values less than 0.05, were produced by PS cutpoints between 0 and 20%, IS cutpoints of 1+ or 2+, and HS cutpoints between 0 and 40. Hazard ratios produced by higher cutpoints were not statistically significant, indicating that patients with very high EGFR expression did not experience enhanced survival benefit from erlotinib treatment compared with placebo. In fact, the hazard ratios tended to increase (indicating less treatment benefit) with increasing EGFR expression.

Hazard ratios and corresponding p values for patients with EGFR-negative tumors using every possible cutpoint are also displayed in Table 3 for each EGFR measure. These hazard ratios were also generally less than 1.0 regardless of the cutpoint used to define EGFR positivity, indicating a survival benefit for patients with EGFR-negative tumors who received erlotinib compared with placebo. Hazard ratios that were statistically significantly less than 1.0, with unadjusted p values < 0.05, were produced when relatively high cutoffs

TABLE 2A. Cutpoint Analysis to Assess Prognostic Effect of Proportion Score (PS)

	Placebo arm (n = 115)				Erlotinib arm (n = 210)			
PS cutpoint	% positive	HR EGFR+: EGFR−	95% CI	Log rank p value	% positive	HR EGFR+: EGFR−	95% CI	Log rank p value
≥1%	71	1.317	0.840–2.066	0.2272	70	0.955	0.683–1.335	0.7877
≥2%	70	1.317	0.849–2.045	0.2170	65	0.839	0.608–1.158	0.2854
≥5%	67	1.249	0.815–1.916	0.3052	63	0.879	0.639–1.210	0.4292
≥10%	58	1.166	0.778–1.748	0.4564	56	0.868	0.636–1.184	0.3705
≥15%	46	1.137	0.763–1.695	0.5267	47	0.877	0.645–1.192	0.4017
≥20%	46	1.137	0.763–1.695	0.5267	45	0.910	0.669–1.238	0.5465
≥25%	40	1.026	0.683–1.543	0.9013	38	0.874	0.637–1.198	0.4019
≥30%	40	1.026	0.683–1.543	0.9013	36	0.952	0.694–1.308	0.7631
≥40%	31	1.048	0.683–1.606	0.8325	32	1.035	0.747–1.434	0.8360
≥50%	29	1.081	0.696–1.678	0.7286	30	1.077	0.774–1.498	0.6591
≥60%	23	1.087	0.675–1.749	0.7313	24	1.119	0.787–1.591	0.5325
≥70%	17	1.191	0.713–1.989	0.5041	20	1.114	0.762–1.627	0.5775
≥75%	13	1.238	0.701–2.187	0.4607	17	1.024	0.677–1.549	0.9092
≥80%	12	1.159	0.644–2.083	0.6217	15	1.133	0.745–1.724	0.5598
≥85%	10	1.254	0.667–2.357	0.4812	12	0.997	0.618–1.610	0.9906
≥90%	9	1.146	0.594–2.213	0.6837	12	0.997	0.618–1.610	0.9906
≥95%	5	1.075	0.466–2.482	0.8653	7	1.139	0.632–2.054	0.6647

TABLE 2B. Cutpoint Analysis to Assess Prognostic Effect of Intensity Score (IS)

	Placebo arm (n = 115)				Erlotinib arm (n = 210)			
IS cutpoint	% positive	HR EGFR+: EGFR−	95% CI	Log rank p value	% positive	HR EGFR+: EGFR−	95% CI	Log rank p value
≥1+	71	1.317	0.840–2.066	0.2272	70	0.955	0.683–1.335	0.7877
≥2+	50	1.099	0.739–1.635	0.6408	46	0.920	0.676–1.251	0.5944
≥3+	28	1.209	0.785–1.861	0.3874	22	1.204	0.837–1.733	0.3174

TABLE 2C. Cutpoint Analysis to Assess Prognostic Effect of Hybrid Score (HS)

	Placebo arm (n = 115)				Erlotinib arm (n = 210)			
HS cutpoint	% positive	HR EGFR+: EGFR−	95% CI	Log rank p value	% positive	HR EGFR+: EGFR−	95% CI	Log rank p value
≥2	71	1.317	0.840–2.066	0.2272	70	0.955	0.683–1.335	0.7877
≥3	70	1.317	0.849–2.045	0.2170	66	0.810	0.586–1.118	0.1999
≥4	70	1.317	0.849–2.045	0.2170	65	0.839	0.608–1.158	0.2854
≥6	68	1.226	0.797–1.886	0.3522	63	0.879	0.639–1.210	0.4292
≥10	67	1.249	0.815–1.916	0.3052	63	0.879	0.639–1.210	0.4292
≥15	61	1.147	0.761–1.729	0.5101	57	0.900	0.659–1.228	0.5063
≥20	60	1.099	0.731–1.652	0.6500	56	0.868	0.636–1.184	0.3705
≥30	53	1.195	0.801–1.782	0.3802	50	0.862	0.634–1.172	0.3433
≥40	49	1.210	0.812–1.803	0.3462	46	0.898	0.660–1.221	0.4929
≥45	44	1.083	0.725–1.616	0.6967	44	0.935	0.687–1.272	0.6669
≥60	44	1.083	0.725–1.616	0.6967	44	0.968	0.711–1.318	0.8377
≥75	41	0.989	0.659–1.484	0.9573	39	0.973	0.712–1.330	0.8657
≥80	41	0.989	0.659–1.484	0.9573	38	1.028	0.752–1.407	0.8606
≥90	37	0.963	0.636–1.457	0.8567	36	0.952	0.693–1.309	0.7638
≥100	35	1.009	0.664–1.534	0.9652	34	0.975	0.705–1.347	0.8770
≥120	33	0.993	0.649–1.518	0.9735	32	0.970	0.699–1.347	0.8562
≥140	27	1.184	0.758–1.849	0.4556	29	1.032	0.737–1.446	0.8543
≥150	27	1.184	0.758–1.849	0.4556	28	1.033	0.736–1.451	0.8504
≥160	25	1.285	0.818–2.017	0.2741	25	1.083	0.762–1.540	0.6557
≥180	24	1.289	0.816–2.035	0.2740	25	1.083	0.762–1.540	0.6557
≥200	22	1.347	0.842–2.155	0.2121	23	1.075	0.747–1.547	0.6975
≥210	19	1.287	0.786–2.109	0.3142	21	1.112	0.767–1.612	0.5759
≥225	17	1.297	0.776–2.169	0.3194	20	1.157	0.792–1.690	0.4510
≥240	17	1.230	0.727–2.080	0.4388	19	1.270	0.866–1.862	0.2219
≥270	12	1.193	0.664–2.144	0.5540	16	1.321	0.873–1.998	0.1880
≥280	12	1.193	0.664–2.144	0.5540	15	1.276	0.838–1.942	0.2561
≥320	9	1.209	0.626–2.337	0.5703	13	1.059	0.669–1.675	0.8079
≥340	9	1.209	0.626–2.337	0.5703	11	0.907	0.548–1.499	0.7019
≥360	8	1.096	0.550–2.185	0.7943	11	0.907	0.548–1.499	0.7019
≥380	4	0.989	0.397–2.466	0.9813	7	1.069	0.579–1.974	0.8313

EGFR, epidermal growth factor receptor; HR, hazard ratio for death; CI, confidence interval.

were used. Because use of high cutoffs to define EGFR positivity produces subsets of EGFR-negative patients whose tumors contain relatively high levels of EGFR expression, this finding should not be surprising. When low cutoffs were used, hazard ratios for EGFR-negative patients increased and were not significantly different than 1.0.

Hazard ratios for patients with EGFR-positive tumors by IHC tended to be less than those for patients with EGFR-negative tumors, suggesting greater benefit from erlotinib among patients with EGFR-positive tumors. To formally compare hazard ratios between EGFR-positive and EGFR-negative patients, statistical tests of interactions between EGFR status and treatment were performed. p values from these interaction tests are displayed in the last column of Table 3. None of the interaction p values were statistically significant. Use of any staining to define EGFR positivity produced hazard ratios and interaction p values that were about the same as those produced when ≥10% staining was

TABLE 3A. Cutpoint Analysis to Assess Predictive Effect of Proportion Score (PS)

		EGFR positive		EGFR negative		
PS cutpoint	% positive	HR erlotinib: placebo	*p* value for treatment	HR erlotinib: placebo	*p* value for treatment	*p* value for interaction
≥1%	70	0.697	0.0158	0.957	0.8579	0.2616
≥2%	67	0.665	0.0079	1.043	0.8537	0.1097
≥5%	65	0.681	0.0142	0.977	0.9171	0.2003
≥10%	57	0.678	0.0216	0.926	0.6965	0.2503
≥15%	46	0.656	0.0241	0.883	0.4766	0.2839
≥20%	46	0.667	0.0314	0.864	0.4012	0.3525
≥25%	38	0.696	0.0775	0.820	0.2243	0.5349
≥30%	38	0.728	0.1234	0.793	0.1551	0.7604
≥40%	32	0.766	0.2389	0.760	0.0743	0.9598
≥50%	30	0.779	0.2903	0.756	0.0652	0.9438
≥60%	24	0.823	0.4661	0.752	0.0496	0.8843
≥70%	19	0.743	0.3149	0.769	0.0630	0.8034
≥75%	15	0.684	0.2589	0.780	0.0714	0.5678
≥80%	14	0.795	0.5086	0.761	0.0466	0.9170
≥85%	11	0.655	0.2729	0.779	0.0638	0.5646
≥90%	11	0.713	0.3966	0.772	0.0545	0.7191
≥95%	6	1.108	0.8629	0.753	0.0307	0.8376

TABLE 3B. Cutpoint Analysis to Assess Predictive Effect of Intensity Score (IS)

		EGFR positive		EGFR negative		
IS cutpoint	% positive	HR erlotinib: placebo	*p* value for treatment	HR erlotinib: placebo	*p* value for treatment	*p* value for interaction
≥1+	70	0.697	0.0158	0.957	0.8579	0.2616
≥2+	47	0.699	0.0499	0.843	0.3401	0.4449
≥3+	24	0.783	0.3190	0.771	0.0818	0.9895

used to define EGFR positivity ($p = 0.2616$ and 0.2503, respectively). Both of these definitions seem to be superior to high HS cutoffs, such as HS ≥ 200 as proposed by Hirsch[17] and Cappuzzo.[18]

These results suggest that the treatment benefits from erlotinib relative to placebo are not significantly different for patients with EGFR-positive and EGFR-negative tumors as determined by IHC, regardless of the EGFR measure or the cutoff used to define EGFR positivity.

Despite the nonsignificant interaction between EGFR protein expression status and treatment, the HR for EGFR-negative patients, using ≥10% staining to define EGFR positivity, was 0.926 ($p = 0.6965$), which highly suggests a lack of treatment benefit in this subset of patients. However, there is evidence that some patients with apparently EGFR-negative tumors did benefit from erlotinib treatment (Table 4). For example, the hazard ratio for patients with Eastern Cooperative Oncology Group performance status 0 or 1 who had received only one previous chemotherapy regimen was 0.47 ($p = 0.02$). Median survivals were 9.0 and 5.3 months, respectively, for the erlotinib and placebo arms. This subset comprised 35% of all patients with tumors with staining in less than 10% of tumor cells. Among patients with tumors with staining in less than 10% of tumor cells who received erlotinib, 72% experienced rash. The median survival of these patients was 8.6 months, compared with 6.2 months for patients with EGFR-negative tumors who received placebo (HR = 0.68, $p = 0.08$). Therefore, EGFR-negativity as determined by IHC, as a single biomarker, does not necessarily predict failure to benefit from treatment with erlotinib.

DISCUSSION

The majority of publications that describe the clinical utility of EGFR status do not contain a control group from a randomized clinical trial. Therefore, statements about the clinical significance of EGFR status are confounded by prognostic significance and predictive significance as defined in this study. NCIC CTG Study BR.21 provided an opportunity to thoroughly assess both the prognostic significance of EGFR expression by IHC and its ability to differentially predict survival of patients with advanced NSCLC who received either erlotinib or placebo.

In this study, EGFR protein expression by IHC was not a significant prognostic factor for survival of patients in the placebo arm. Despite a suggestion that patients with EGFR-positive tumors had slightly worse survival than patients with

TABLE 3C. Cutpoint Analysis to Assess Predictive Effect of Hybrid Score (HS)

		EGFR positive		EGFR negative		
HS cutpoint	**% positive**	**HR erlotinib: placebo**	***p* value for treatment**	**HR erlotinib: placebo**	***p* value for treatment**	***p* value for interaction**
≥2	70	0.697	0.0158	0.957	0.8579	0.2616
≥3	67	0.658	0.0062	1.061	0.7982	0.0848
≥4	67	0.665	0.0079	1.043	0.8537	0.1097
≥6	65	0.686	0.0156	0.963	0.8683	0.2282
≥10	65	0.681	0.0142	0.977	0.9171	0.2003
≥15	58	0.706	0.0354	0.910	0.6422	0.3640
≥20	57	0.706	0.0369	0.903	0.6103	0.3773
≥30	51	0.640	0.0118	0.926	0.6797	0.1716
≥40	47	0.644	0.0166	0.896	0.5387	0.2206
≥45	44	0.697	0.0593	0.832	0.2864	0.5309
≥60	44	0.709	0.0730	0.820	0.2487	0.6215
≥75	40	0.753	0.1592	0.782	0.1378	0.9146
≥80	39	0.775	0.2077	0.766	0.1071	0.9283
≥90	36	0.763	0.2017	0.774	0.1095	0.9537
≥100	34	0.758	0.2052	0.766	0.0895	0.9732
≥120	32	0.772	0.2522	0.760	0.0757	0.9711
≥140	28	0.704	0.1459	0.782	0.1010	0.6707
≥150	28	0.707	0.1525	0.781	0.0993	0.6754
≥160	25	0.678	0.1206	0.791	0.1118	0.5607
≥180	25	0.681	0.1298	0.789	0.1071	0.5604
≥200	22	0.637	0.0887	0.797	0.1186	0.4382
≥210	21	0.692	0.1926	0.781	0.0831	0.6117
≥225	19	0.729	0.2849	0.770	0.0641	0.7184
≥240	18	0.822	0.5172	0.750	0.0412	0.9301
≥270	14	0.859	0.6566	0.743	0.0303	0.7670
≥280	14	0.836	0.6034	0.747	0.0338	0.8412
≥320	11	0.726	0.4151	0.769	0.0510	0.7456
≥340	10	0.635	0.2644	0.783	0.0679	0.4961
≥360	10	0.697	0.3925	0.776	0.0581	0.6545
≥380	6	1.278	0.7172	0.752	0.0297	0.8056

EGFR, epidermal growth factor receptor; HR, hazard ratio for death.

TABLE 4. Subsets of Patients with EGFR-Negative Tumors (Staining < 10%)

Subset	**Percentage of patients with staining < 10%**	**HR**	***p* value**
Second line	48%	0.63	0.09
ECOG PS 0 or 1	72%	0.80	0.35
ECOG PS 0 or 1, second line	35%	0.47	0.03
Patients with rash	72% of Tarceva-treated patients with staining < 10%	0.68	0.08

Second line, patients who received only one previous chemotherapy regimen; ECOG PS, Eastern Cooperative Oncology Group performance status.

EGFR-negative tumors, there were no cutpoints for any of the three EGFR measures that produced a statistically significant separation with respect to survival between these subsets of patients. Similarly, in the erlotinib arm, EGFR protein expression by IHC was not a significant prognostic factor for survival despite a suggestion that patients with EGFR-positive tumors had slightly better survival. There may, however, be a selection bias in any clinical trial that includes patients who have received prior therapy for their disease. It is possible that EGFR status is a strong prognostic factor for patients with newly diagnosed NSCLC and that patients with EGFR-positive tumors died earlier or were more likely to have been excluded from participation in NCIC CTG Study BR.21. Nevertheless, among patients who were enrolled in

this clinical trial, EGFR protein expression by IHC was not a strong prognostic factor.

One might expect that patients with EGFR-positive tumors should derive greater benefit from therapy with EGFR inhibitors compared with patients with EGFR-negative tumors. Visually, this appeared to the case in NCIC CTG Study BR.21, where the hazard ratio for death for patients with EGFR-positive tumors was 0.678 ($p = 0.0216$), compared with 0.926 ($p = 0.6965$) for patients with EGFR-negative tumors, using the predefined definition of EGFR positivity of staining in ≥10% of tumor cells. Nevertheless, a formal comparison of these hazard ratios indicated that they were not statistically significantly different ($p = 0.2503$).

Evaluation of different measures of EGFR protein expression by IHC and different cutpoints for defining EGFR positivity revealed that EGFR protein expression was a weak predictive factor for survival benefit from erlotinib in this study. The hazard ratios for death were generally lower (indicating more treatment benefit) for patients with EGFR-positive tumors compared with patients with EGFR-negative tumors; nevertheless, none of the interactions between EGFR status and treatment benefit were statistically significant. Use of very high cutpoints to define patients with EGFR-rich tumors that might be especially sensitive to treatment with erlotinib cannot be supported by these data. On the contrary, because patients with even low levels of EGFR protein expression may derive some benefit from erlotinib, if a cutpoint must be used, it should be as low as possible. Thus, these data support the recommendation supplied with Dako EGFR pharmDx™ kits (DakoCytomation California Inc, Carpinteria, CA) that any membrane staining should be interpreted as EGFR positive.

There are several possible explanations for lack of correlations between EGFR protein expression by IHC and clinical outcomes. These include false-negative results attributable to a lack of sensitivity in the detection system, heterogeneity of EGFR expression within the tumor, and specific mutations that might mediate responses to the tyrosine kinase inhibitors.[26] Most of the tumor samples in this study were obtained at the time of diagnosis, and all patients received and eventually failed intervening first-line therapy. It is not clear whether EGFR protein expression as determined by IHC is affected by such therapy. Emerging data indicate that EGFR protein expression in primary tumors may not correlate with EGFR expression in metastatic sites, at least in colorectal tumors.[27] In addition, expression of EGFR may have different consequences depending on histological subtype and coexpression of TGFα and ras genotype.[28] It is possible that some of the tumors were not adequate to accurately reflect the status of protein expression by IHC. Although all tumor samples were visually examined before they were assayed by IHC, a specific protocol that included requirements for minimum numbers of cells evaluated, minimum numbers of blocks/sections to be evaluated, type and duration of fixation, and site of biopsy was not used. Clearly, if a sample is inadequate, no molecular test will give informative results, regardless of how good or relevant the measured endpoint might be. Thus, the failure to find statistically significant relationships between EGFR protein expression and survival might be attributable to the quality of the samples and/or the sensitivity of the assay.

It is also possible that the relatively modest sample size of 325 patients with evaluable EGFR protein expression results lacked sufficient statistical power to detect meaningful prognostic or predictive relationships. Nevertheless, given the multiplicity of statistical tests that were performed, one might have expected a few cutpoints to have demonstrated statistical significance by chance alone. There is a considerable literature that warns of overinterpretation of results from searches for "optimal" cutpoints, with suggestions to adjust p values to minimize the chances of falsely claiming statistical significance.[29,30] The p values that are reported in Tables 2 and 3 are undoubtedly overly optimistic and should be inflated before they can be properly interpreted.

Comparisons across studies are difficult because different EGFR antibodies, preparation regimens, and scoring systems could contribute to discrepancies between studies. Variations in fixatives and storage time of tissue sections also can impact the interpretation of assay results.[31]

Cetuximab (ImClone Systems, Inc., New York, NY) is a monoclonal antibody that targets the extracellular domain of EGFR. It is approved for the treatment of advanced colorectal cancer for patients with tumors that express EGFR as measured by the same Dako EGFR pharmDx kits used in NCIC CTG Study BR.21. Recently, clinical activity has been demonstrated in patients with colorectal cancer whose tumors were scored as EGFR negative by IHC.[32] These tumors may well have expressed EGFR protein that was not detected by the assay. The authors concluded that selection or exclusion of patients for cetuximab therapy on the basis of currently available EGFR IHC tests is not warranted.

Based on the results of the current study, a similar conclusion might be appropriate for erlotinib and patients with NSCLC. Although the entire subset of patients with EGFR-negative tumors as determined by IHC did not seem to benefit from treatment with erlotinib, relatively large subsets of patients with apparently EGFR-negative tumors did benefit in this study. This suggests that additional factors should be taken into account before treatment decisions can be made. It is possible that EGFR protein expression is truly an important prognostic and predictive biomarker but that current techniques for performing IHC testing are not capable of discriminating in this regard.

It has been suggested that total EGFR expressed on the cell membrane may not be the most relevant biomarker for predicting responses to EGFR tyrosine kinase inhibitors. Because the effects of EGFR activation on the cell are mediated by downstream signal-transduction cascades, including the PI3K/Akt, RAS/Raf/Erk, and Jak/STAT pathways, EGFR downstream molecules have also been investigated.[33–37] Results of these investigations have generally been disappointing in that relationships between these biomarkers and clinical outcomes have not been consistently observed. More recently, amplification or high polysomy of the EGFR gene has shown promise as a predictor of clinical benefit from EGFR inhibitors,[13,17,18,25] and several studies are underway to confirm these results.

One might anticipate that EGFR protein expression determined by IHC in combination with other biomarkers in the EGFR pathway might eventually provide a means for identifying patients who should or should not be considered candidates for treatment with EGFR inhibitors. This may be particularly important in earlier stages of disease, where first-line treatment and adjuvant therapy are commonly administered. While we wait for these results, it might be prudent to adopt a more pragmatic approach for patient selection in the second- and third-line settings.

Treatment-related rash has been associated with relatively long survival in a number of studies involving EGFR inhibitors. In NCIC CTG Study BR.21, the median time to develop rash was 8 days, and 90% of patients who experienced rash did so within 25 days. Because 72% of the patients with EGFR-negative tumors by IHC who received erlotinib in NCIC CTG Study BR.21 experienced rash, and their median survival was 8.6 months compared with a median of 5.3 months for EGFR-negative patients who received placebo, it might be reasonable to consider all patients with incurable stage IIIB/IV NSCLC who have failed standard therapy for advanced or metastatic disease to be candidates for treatment with erlotinib, regardless of their EGFR protein expression status by IHC. Cumulative evidence from this and other studies suggests that patients who do not experience rash within 3 to 4 weeks may have poor clinical outcomes. Studies exploring this further are being conducted and include testing of dose intensification in patients who do not experience rash. Such patients may be candidates for alternative therapies.

REFERENCES

1. Nicholson RI, Gee JMW, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001;37:S9–S15.
2. Veale D, Kerr N, Gibson GJ, et al. The relationship of quantitative epidermal growth factor receptor expression in non-small cell lung cancer to long term survival. *Br J Cancer* 1993;68:162–165.
3. Ohsaki Y, Tanno S, Fujita Y, et al. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. *Oncol Rep* 2000;7:603–607.
4. Meert AP, Martin B, Verdebout JM, et al. Is there a relationship between c-erbB-1 and c-erbB-2 amplification and protein overexpression in NSCLC? *Lung Cancer* 2006;47:325–336.
5. Meert AP, Martin B, Delmotte P, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. *Eur Respir J* 2002;20:975–981.
6. Dazzi H, Hasleton PS, Thatcher N, et al. Expression of epidermal growth factor receptor (EGF-R) in non small cell lung cancer. Use of archival tissue and correlation of EGF-R with histology, tumour size, node status and survival. *Br J Cancer* 1989;59:746–749.
7. Pastorino U, Andreola S, Tagliabue E, et al. Immunocytochemical markers in stage I lung cancer: relevance to prognosis. *J Clin Oncol* 1997;15:2858–2865.
8. Berghmans T, Meert AP, Martin B, et al. Prognostic role of epidermal growth factor receptor in stage III nonsmall cell lung cancer. *Eur Respir J* 2006;25:329–335.
9. Bailey R, Kris M, Wolf M, et al. Gefitinib (Iressa, ZD 1839) monotherapy for pretreated advanced non-small-cell lung cancer in IDEAL 1 and 2; tumor response is not clinically relevantly predictable from tumor EGFR membrane staining alone (Abstract). *Lung Cancer* 2003;41:s71.
10. Cortes-Funes H, Parra HS. Extensive experience of disease control with gefitinib and the role of prognostic markers. *Br J Cancer* 2003;89(Suppl 2):S3–S8.
11. Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. *J Clin Oncol* 2003;21:2658–2663.
12. Parra HS, Cavina R, Latteri F, et al. Analysis of epidermal growth factor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. *Br J Cancer* 2004;91:208–212.
13. Hirsch F, Varella-Garcia M, Dziadziuszko R, et al. Molecular analysis of EGFR gene copy number, EGFR expression and Akt activation status in advanced non-small cell lung cancer treated with gefitinib or placebo (ISEL trial). AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics. Philadelphia, PA, 2006.
14. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J Clin Oncol* 2004;22:3238–3247.
15. Gatzemeier U, Pluzanska A, Szczesna A, et al. Results of a phase III trial of erlotinib (OSI-774) combined with cisplatin and gemcitabine (GC) chemotherapy in advanced non-small cell lung cancer (NSCLC) (Abstract). *Proc Am Soc Clin Oncol* 2004;22:617.
16. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: A phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* 2006;23:5892–5899.
17. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003;21:3798–3807.
18. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2006;97:643–655.
19. Clark GM. Do we really need prognostic factors for breast cancer? *Breast Cancer Res Treat* 1994;30:117–126.
20. Clark GM. Interpreting and integrating risk factors for patients with primary breast cancer. *J Natl Cancer Inst Monographs* 2001;30:17–21.
21. Hayes DF, Trock B, Harris AL. Assessing the clinical impact of prognostic factors: when is "statistically significant" clinically useful? *Breast Cancer Res Treat* 1998;52:305–319.
22. U.S. Food and Drug Administration. Tarceva® (erlotinib tablets). Washington, DC: US Government Printing Office; 2004:1–21.
23. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2006;353:123–132.
24. EGFR pharmDx™ Interpretation Manual. Available at http://www.dakogmbh.de/egfr_interpretation_manual.pdf. Accessed April 7, 2006.
25. Tsao M-S, Sakurada A, Cutz J-C, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2006;353:133–144.
26. Dei Tos AP, Ellis I. Assessing epidermal growth factor receptor expression in tumours: what is the value of current test methods? *Eur J Cancer* 2006;41:1383–1392.
27. Scartozzi M, Bearzi I, Berardi R, et al. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. *J Clin Oncol* 2004;23:4720–4726.
28. Hsieh ET, Shepherd FA, Tsao MS. Co-expression of epidermal growth factor receptor and transforming growth factor-alpha is independent of ras mutations in lung adenocarcinoma. *Lung Cancer* 2000;29:151–157.
29. Hilsenbeck SG, Clark GM, McGuire WL. Why do so many prognostic factors fail to pan out? *Breast Cancer Res Treat* 1992;22:197–206.
30. Altman DG, Lausen B, Sauerbrei W, et al. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst* 1994;86:829–835.
31. Atkins D, Reiffen KA, Tegtmeier CL, et al. Immunohistochemical detection of EGFR in paraffin-embedded tumor tissues: variation in staining intensity due to choice of fixative and storage time of tissue sections. *J Histochem Cytochem* 2004;52:893–901.
32. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol* 2006;23:1803–1810.
33. Han SW, Hwang PG, Chung DH, et al. Epidermal growth factor receptor (EGFR) downstream molecules as response predictive markers for gefitinib (Iressa, ZD1839) in chemotherapy-resistant non-small cell lung cancer. *Int J Cancer* 2006;113:109–115.

34. Cappuzzo F, Magrini E, Ceresoli GL, et al. Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. *J Natl Cancer Inst* 2004;96:1133–1141.
35. Pao W, Zakowski M, Cordon-Cardo C, et al. Molecular characteristics of non-small cell lung cancer (NSCLC) patients sensitive to gefitinib (Abstract). *Proc Am Soc Clin Oncol* 2004;23:619.
36. Franklin WA, Chansky K, Gumerlock PH, et al. Association between activation of ErbB pathway genes and survival following gefitinib treatment in advanced BAC (SWOG 0126) (Abstract). *Proc Am Soc Clin Oncol* 2004;23:618.
37. Han S-W, Kim T-Y, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006;11: 2493–2501.